Bioaccessibility of phenolic compounds in common beans (*Phaseolus vulgaris L.*) after *in vitro* gastrointestinal digestion: a comparison of two cooking procedures

Alicia P. Cárdenas-Castro[1], Jara Pérez-Jiménez[2], Luis A. Bello-Pérez[3], Juscelino Tovar[4], Sonia G. Sáyago-Ayerdi[1]*

[1]Tecnológico Nacional de México/ Instituto Tecnológico de Tepic, Laboratorio Integral de Investigación en Alimentos, División de Estudios de Posgrado, Av. Tecnológico No 2595, Col. Lagos del Country CP 63175, Tepic, Nayarit México.

[2] Institute of Food Science Technology and Nutrition (ICTAN-CSIC), Jose Antonio Novais 10, 28040, Madrid, Spain

[3] Instituto Politécnico Nacional, CEPROBI, Km. 6.6 Carr. Yautepec-Jojutla Col. San Isidro, 62731, Yautepec, Morelos, México

[4] Department of Food Technology, Engineering and Nutrition, Lund University. P.O. Box 124, SE-221 00, Lund, Sweden

*Corresponding author, e-mail: sonia.sayago@gmail.com; ssayago@ittepic.edu.mx

Tel.: +52 311 211 94 00 ext 328

Abstract

Background and objectives: Beans (*Phaseolus vulgaris* L.) are widely consumed, but the bioaccessibility of their phenolic compounds (PC) may be affected by different factors. ~~such as the cooking method applied.~~ Within this framework, an *in vitro* gastrointestinal digestion of two beans varieties: ´Azufrado´ and ´Negro Jamapa´, was performed and the bioaccessibility and *in vitro* release kinetics of PC was evaluated. Mashed beans were prepared by two common culinary procedures in Mexico: pressure cooking followed by mashing, or pressure cooking and mashing, followed by frying.

Findings: ~~A decrease in the antioxidant capacity (AOX) values was observed for the cooked-fried samples.~~ The bioaccessibility of PC was about 50% in the cooked samples, and 30% in cooked-fried samples. The cooking condition did not modify the PC release kinetics during the first 60 min in any of the bean preparations. Three PC were identified by HPLC-DAD-MS: kaempferol-3-*O*-glucoside, quercetin-3-*O*-glucoside, and chlorogenic acid, which was the main PC released from all samples.

Conclusions: Simulated gastrointestinal digestion revealed processing-related differences in the PC bioaccessibility in these two bean varieties, which should be further considered and evaluated in nutritional studies.

Significance and novelty: The study is in line with current approaches for assessing PC bioaccessibility during the gastrointestinal digestion, providing knowledge on the types and quantities of PC released from the food matrix of beans as eaten.

Keywords

Beans; *in vitro* gastrointestinal digestion; bioaccessibility; kinetics; phenolic compounds

1 INTRODUCTION

One of the most important staple foods in the Mexican diet are common beans (*Phaseolus vulgaris* L.). Beans have an important scientific interest due to their macronutrient and micronutrient profile; moreover, they contain several types of bioactive compounds (BC), such as flavonoids, which constitute the major group of phenolic compounds (PC) in beans (Chávez-Mendoza & Sánchez, 2017). *In vitro* models have shown potential biological effects of PC extracted from beans, particularly in relation to cancer, as they affect cell proliferation (Moreno-Jiménez et al., 2015). Nevertheless, studies that analyze beans as they are eaten after different cooking processes are scarce. Cooking promotes changes in the nutritional composition, including the BC contents (Silva-Cristobal, Osorio-Díaz, Tovar, & Bello-Pérez, 2010).

Another important aspect regarding the bioactive potential of common beans relates to the fact that BC must be bioaccessible during digestion (released from the food matrix) and then bioavailable (absorbed in the small intestine) in order to exert health-beneficial effects (Shahidi, Varatharajan, Oh, & Peng, 2019). Specifically, the interaction between macronutrients and dietary fiber with PC has been reported, involving covalent bonds, non-covalent hydrophobic interactions and hydrogen bonding, all influencing the bioaccessibility and bioavailability of PC during digestion (Jakobek, 2015). Thus, *in vitro* simulating human digestion models are useful tools to determine the bioaccessibility of BC. In regard to beans, little is known about the PC that are released and potentially absorbed in the human gut. Neither is clear which ones remain unaltered after the human gastrointestinal process.

The aim of this study was to assess the bioaccessibility and the kinetics of release of PC after *in vitro* gastrointestinal digestion in cooked common beans. Two agronomic varieties

of beans: ´Azufrado´ and ´Negro Jamapa´, and two preparation procedures, i.e. pressure cooking alone or combined with frying, were evaluated.

2 MATERIALS AND METHODS

2.1 Sample preparation

Two *P. vulgaris* L. varieties, ´Azufrado´ and ´Negro Jamapa´, were provided by Cadena Agroalimentaria de Frijol de Nayarit A.C. The seeds were washed using a 1/3 (w/v) bean/water ratio. The processing was performed according to two traditional culinary customs for the preparation of beans. The seeds were cooked in a pressure cooker for the time necessary to obtain soft seeds -similar to the "traditionally" prepared beans- according to the finger compression test (15 psi for 7 min and 9 min for 'Azufrado' and 'Negro Jamapa' varieties, respectively, Presto, 77235, Mexico), and subsequently mashed at slow speed using a 2-speed immersion hand blender with steel shaft stainless (Oster, 2614, Mexico). These samples were named as: cooked-mashed ´Azufrado´ (CA), and cooked-mashed ´Negro Jamapa´ (CN). Subsequently, half of the mashed material was fried for 4 min in a deep stainless-steel pan (Tramontina, Everyday, Mexico) using a bean/vegetable oil ratio of 1/6 (w/v), obtaining the: 'cooked-mashed-fried´'Azufrado' (CFA) and 'cooked-mashed-fried´ ´Negro Jamapa´ (CFN) samples. All samples were frozen at -80 °C and freeze-dried (FreeZone 6, Labconco, USA) for chemical composition and indigestible fraction analyses.

2.2 Chemical composition

The four samples were subjected to chemical composition analysis using AOAC methods (AOAC, 2005) for moisture (Method 925.10), fat (Method 920.39), protein (Method 920.87), and ash (923.03). Available starch (AS) content was quantified following a multienzymatic protocol (Holm, Björck, Drews, & Asp, 1986) using Termamyl® (Novo

93 A/S, Copenhagen) and amyloglucosidase (A-9913, Sigma-Aldrich, St. Louis, MO, USA).
94 The resistant starch (RS) content was measured as the enzymatically non-hydrolyzable
95 starch fraction (Goñi, García-Diz, Mañas, & Saura-Calixto, 1996).

96 **2.3 Analysis of total indigestible fraction (TIF)**

97 The samples (CA, CN, CFA, CFN) were submitted to simulated physiological conditions
98 of human digestion, according to the method proposed by Saura-Calixto, García-Alonso,
99 Goñi, and Bravo (2000), which includes enzymatic treatments with pepsin (0.2 mL of a 300
100 mg/mL solution in HCl-KCl 0.2 M buffer, pH 1.5, 40 °C, 1 h, P-7000, Sigma-Aldrich),
101 pancreatin (1 mL of 5 mg/mL solution in phosphate buffer 0.1 M; pH 7.5, 37 °C, 6 h, P-
102 1750, Sigma-Aldrich), and α-amylase (1 mL of a 120 mg/mL solution in tris maleate buffer
103 0.1 M; pH 6.9, 37 °C, 16 h, A-3176, Sigma-Aldrich). After the enzymatic hydrolysis, the
104 samples were centrifuged (15 min, 25 °C, 3000 rpm) and supernatants were collected. The
105 residues were considered as the insoluble indigestible fraction (IIF) and quantified
106 gravimetrically. Supernatants were incubated (45 min, 60 °C) with 100 µL
107 amyloglucosidase (A-9913, Sigma-Aldrich), transferred into dialysis tubes (D9652-30.48 m
108 avg, Flat width 33 mm), 12,400 Da, Sigma-Aldrich) and dialyzed against water for 48 h at
109 25 °C (water flow 7 L/h). The dialyzed supernatants (solution inside the dialysis tubes)
110 constitute the soluble indigestible fraction (SIF). A SIF aliquot was hydrolyzed with 1 M
111 sulfuric acid (100 °C for 90 min) for estimating SIF content, after reaction with
112 dinitrosalicylic acid. TIF was quantified as the sum of IIF + SIF, and results were expressed
113 as g/100g DW.

114 **2.4 PC analysis**

115 An organic aqueous extraction of the samples (CA, CN, CFA, and CFN) was performed
116 following the protocol by Pérez-Jiménez et al. (2008), obtaining a supernatant, containing

117 total soluble polyphenols (TSP). TSP content was evaluated in the supernatants of the
118 aqueous extract using a microplate reader (Biotek® Synergy HT, USA) with Gen5
119 software, using a calibration curve made with gallic acid read and at 750 nm (Montreau,
120 1972; Alvarez-Parrilla, de la Rosa, Amarowics, & Shahidi, 2010). Results were expressed
121 as mg GAE/g DW. The residue of the extraction contained the non-extractable polyphenols
122 (NEPP), which are divided in two classes: hydrolyzable polyphenols (HP) and non-
123 extractable proanthocyanidins (NEPA). For HP quantification, the residues were submitted
124 to Hartzfeld, Forkner, Hunter, and Hagerman (2002) protocol, followed by Folin-
125 Ciocalteau assay as described above, while for NEPA, the Reed, McDowell, Van Soest, and
126 Horvarth (1982) protocol was applied. The concentration of HP was calculated with a gallic
127 acid calibration curve (mg GAE/g DW), and the NEPA content was calculated using a
128 calibration curve prepared with carob pod proanthocyanidins. Results were expressed as
129 mg/g DW.

130 **2.5 Antioxidant capacity (AOX)**

131 The supernatants from the aqueous organic extraction were submitted to perform FRAP
132 (ferric reducing/antioxidant power) and ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-
133 sulphonic acid) assay. These analyses were conducted with the modifications described by
134 Alvarez-Parrilla et al. (2010) using a microplate reader (Biotek® Synergy HT, USA) with
135 Gen5 software. The results were expressed as mmol of Trolox (6-hydroxy-2,5,7,8-
136 tetramethylchromane-2-carboxylic) equivalents (mmol TE/g dry weight, DW).

137 **2.6 *In vitro* gastrointestinal digestion process**

138 **2.6.1 *In vitro* bioaccessibility of PC**

139 Six healthy volunteers (mean age 23.2 ± 8.8 and body mass index 21.0 ± 2.3 kg/m^2)
140 participated in the *in vitro* chewing assay. This is a standard non-invasive and accepted

procedure, and the volunteers were not exposed to health risks (Granfeldt, Björck, Drews, & Tovar, 1992; Woolnough, Bird, Monro, & Brennan, 2010). Subjects chewed 1 g of fresh sample of each treatment (CA, CN, CFA and CFN) in fasting stage. Then, the samples were subjected to an *in vitro* digestion model, adapted from reported protocols (Saura-Calixto et al., 2010; Granfeldt et al., 1992) (Fig. 1). Briefly, the sample was chewed by each volunteer during 30 s and expectorated into a beaker with HCl-KCl buffer (pH 1.5) (Step 1). Immediately afterwards, each subject rinsed their mouth with 5 mL of sodium phosphate buffer (0.05 M, pH 6.9 with 0.4 g/L NaCl) and expectorated into the same beaker containing the chewed sample, which was incubated with pepsin (300 mg/mL, pH 1.5, 37°C, 2 h, P-7000, Sigma-Aldrich) in a shaking water bath to simulate the gastric digestion. Subsequently, an aliquot of 2 mL was taken to quantify the PC content according to the TSP (Montreau, 1972; Alvarez-Parrilla et al., 2010) protocols; PC released at this stage represent those present in the gastric fraction (GasF) (Step 2). The next step (Step 3) consisted in digestion with pancreatin (5 g/L, 37 °C, P-1750, Sigma-Aldrich) and pancreatic α-amylase (120 g/L, 37 °C, A-6255, Sigma-Aldrich) in phosphate buffer (0.1 M, pH 6.9), incubated in a shaking water bath in order to simulate intestinal digestion. In the case of fried samples, lipase (7 g/L, L-3126, Sigma-Aldrich) and bile salts (7 g/L, B-8631, Sigma-Aldrich) were also added to the intestinal digestion media. Aliquots of 2 mL were taken to quantify the PC content, according to the TSP (Montreau, 1972; Alvarez-Parrilla et al., 2010) protocols; PC released in this stage correspond to the intestinal fraction (IntF) (Step 3).

Regarding the evaluation of the *in vitro* bioaccessibility of PC, after Step 3 the samples were centrifuged to separate the soluble indigestible fraction (SIF) and insoluble indigestible fraction (IIF) (Step 4). The supernatant was subjected to dialysis in cellulose

dialysis against tap water in cellulose dialysis bags (D9652, 12-14 KDa, Sigma Aldrich) for 48 h, simulating passive absorption. The residue, containing the PC that are not bioaccessible in the small intestine, was used to determine the PC content associated with IIF. This residue was submitted to an organic aqueous extraction to quantify the TSP (Montreau, 1972; Alvarez-Parrilla et al., 2010) associated to the IIF. Then, the residue of this aqueous organic extraction was submitted to chemical hydrolysis for the quantification of HP (Hartzfeld et al., 2002) and NEPA (Reed et al., 1982). Thus, the total PC associated with IIF was considered as the sum of TSP, HP and NEPA content (Step 5). After the 48 h dialysis time, the supernatant was used to determine the non-bioaccessible PC associated with SIF as TSP (Montreau, 1972; Alvarez-Parrilla et al., 2010) (Step 6). The *in vitro* percentage of the bioaccessibility (%BA) of PC was determined using the following Eq (1):

$$\%BA = \frac{(PC - IntF) - (PC - SIF)}{(PC - IntF) + (PC - IIF)} x100 \qquad \text{Eq (1)}$$

,being PC-IntF= the PC released on the intestinal fraction, PC-SIF= the PC associated with the soluble indigestible fraction, PC-IIF= the PC associated with the insoluble indigestible fraction.

2.6.2 *In vitro* PC release kinetics

The *in vitro* release kinetics was assessed according to the procedure shown in Fig. 1 but after Step 3 the samples were transferred to a cellulose dialysis membrane, and Blancas-Benítez et al. (2015) protocol for HPLC-DAD-MS analysis was followed.

To calculate the kinetic parameters during the *in vitro* release assay, the final rate (*Vf*) of PC release was calculated using the following Eq (2):

$$Vf = \Sigma(\frac{\Delta C}{\Delta t}) \quad \text{Eq (2)}$$

187 Where *Vf* is the final rate of PC release (mg GAE/min), ΔC is the concentration difference
188 among the initial and final PC content and Δt is the time difference among a particular time
189 and the initial time.

190 **2.6.2.1 Identification of PC by HPLC-DAD-MS**

191 The identification of PC was carried out according to a procedure previously reported
192 (Blancas-Benítez, Pérez-Jiménez, Montalvo-González, González-Aguilar, & Sáyago-
193 Ayerdi, 2018) with modifications. For MS assay, a 6120 Agilent simple Quadrupole
194 LC/MS coupled to the HPLC Agilent 1260 series system (Agilent Technologies, Santa
195 Clara, CA, USA), equipped with an electrospray ionization interface in positive and
196 negative ionization was used with the next conditions: drying gas flow (N_2), 13.0 L/min,
197 nebulizer pressure, 40 psi; gas drying temperature, 350°C, and capillary voltage, 4000 V.
198 Samples were injected (10 µL, flow rate 0.5 mL/min) into a Poroshell 120 EC-C18 column
199 (4.6 mm x 150 mm, particle size 2.7 µm). The gradient elution was carried out using water
200 containing 0.1% trifluoroacetic acid (302031, Sigma Aldrich) as solvent A and acetonitrile
201 (Sigma Aldrich) as solvent B. They were applied as follows: 10 min, 74% A, 70 min, 35%
202 A, 71 min, 100% B, 78 min, 90% A. Detection was performed at 280-520 nm. The data
203 analysis was performed using OpenLab CDS, ChemStation Edition software (Agilent
204 Technologies, Santa Clara, CA, USA). The compounds were first detected using a single
205 MS scan in the 100-1500 m/z range based on previously reported *m/z* ions in
206 common/cooked beans, according to the elution time described in the literature followed by
207 a targeted search based on the peaks showing major signals in the UV-Vis chromatograms
208 (Huber, Brigide, Bolis, & Canniatti-Brazaza, 2016; Lin, Harnly, Pastor-Corrales, & Luthria,
209 2008).

210 **2.7 Statistical analysis**

All analyses were performed in triplicate; mean values and standard deviations from each determination were calculated. Depending on the results in the Levene and Shapiro-Wilk tests, data were subjected either to one-way ANOVA/Fisher´s LSD test for all test samples ($p > 0.05$, n=3) or to independent-samples Krustal-Wallis non-parametric test/Multiple comparisons of mean ranks for the release kinetics samples ($p < 0.05$, n=6). All analyses were performed using STATISTICA software, version 10.0 (StatSoft. Inc. 1984-2007, Tulsa, OK, USA).

3 RESULTS AND DISCUSSION

In spite of being a major component of the diet of a large number of people around the world and increasingly recognized as valuable functional foods (~~Amarowicz & Pegg, 2008;~~ Tovar, Nilsson, Johansson, & Björck, 2014), legumes have been mainly studied in terms of the nutritional characteristics of their macronutrient and dietary fiber moieties, while the physiological actions of their BC have been largely overlooked. PC are among those bioactive constituents of legumes that should be investigated in more detail (Chávez-Mendoza & Sánchez, 2017). The present study provides data on bioaccessibility aspects of the PC fraction present in two varieties of common beans as eaten, following two culinary procedures of ample use in Mexico and Central America, i.e. cooking followed by mashing and cooking-mashing combined with pan-frying.

3.1 Chemical composition of cooked and cooked-fried beans

The proximate composition of the samples is shown in Table 1. As expected, the fat content was higher in the cooked-fried samples than in the cooked ones due to the oil retained in the sample during the frying step, with a reduction in moisture content. Significant differences ($p < 0.05$) were found for protein content among the different samples. but it is important to note that the variations in the content of macronutrients are also influenced by

the soil, climate, variety, and collection time (Rehinan, Rashid, & Shah, 2004). Moisture and ash content were significantly lower ($p < 0.05$) in the cooked-mashed-fried samples than in the cooked-mashed samples. Also, it should be considered that a previous mineral loss could take place during the pressure-cooking due to the diffusion of certain minerals to the cooking water (Wang, Hatcher, Tyler, Toews, & Gawalko, 2010). Additionally, starch fractions (AS and RS) and TIF were measured in the samples (Table 1), as food constituents that may affect the bioaccessibility of PC (Jakobek, 2015). The samples did not show differences in AS content. In contrast, the RS content was significantly higher ($p < 0.05$) in cooked-mashed-fried samples, which can be attributed to increased amylose retrogradation (Sáyago-Ayerdi, Tovar, Osorio-Diaz, Paredes-López, & Bello-Pérez, 2005) and/or formation of indigestible amylose/lipid complexes during frying (Bello-Pérez, Flores-Silva, Agama-Acevedo & Tovar, 2019). ´Negro Jamapa´ samples had higher ($p < 0.05$) RS content than ´Azufrado´ samples, both being in the range previously reported for other cooked beans (Sáyago-Ayerdi et al. 2005).

SIF content was similar in all samples, ranging between 4.3 and 4.6%, a lower value than the 10.2% previously reported for cooked common black beans (Hernández-Salazar et al. 2010). This difference may be explained by the chemical hydrolysis of cellulose and hemicellulose (SIF constituents) into glucose, arabinose, xylose, and galactose, during the pressure cooking used in this study. Indeed, Rehinan et al. (2004) reported that pressure cooking (15 psi) originated a 28% decrease in the cellulose content in common red beans and 17% in common white beans, while the content of hemicellulose decreased by 39% in both varieties. In contrast with the lack of differences between the treatments regarding SIF content in both varieties, the IIF content was higher ($p < 0.05$) in cooked-mashed-fried samples than in the cooked-mashed samples. This may be explained by the formation of RS

described above as consequence of the relatively prolonged high temperature exposition of the food during this cooking process (Silva-Cristobal et al. 2010).

3.2 Total PC content and AOX of common cooked beans

The TSP, AOX (ABTS and FRAP assays), HP and NEPA contents are shown in Table 2. Both TSP content and AOX of ´Negro Jamapa´ samples were significantly higher ($p < 0.05$) than those of ´Azufrado´ variety. Nevertheless, it was observed for both varieties that the cooking-mashing-frying treatment tended to result in a lower TSP and AOX values than those samples subjected to cooking-mashing treatment only. Xu and Chang (2010) reported that controlled pressure-cooking allows a better release and preservation of TSP than the conventional cooking process (boiling). Additionally, Gan et al. (2017) reported the total PC content in undigested raw common beans. Our results are similar to this study, which it could be concluded that the cooking process did not affect the PC compared to raw seeds but in contrast, the cooking-mashing-frying procedure used here caused losses in TSP if it is compared with the cooking-mashing process, probably due to changes in the chemical structure –mostly oxidations- associated to the high temperatures applied. Indeed, it has been reported that an aggressive long cooking process may lead to a decrease of BC (Pérez-Burillo, Rufián-Henares, & Pastoriza, 2018). Then, the loss of TSP in cooked-mashed-fried samples can be related to the decrease in AOX values compared with the cooked-mashed samples.

The two categories of NEPP or macromolecular antioxidants, i.e., HP and NEPA, were higher in the ´Negro Jamapa´ samples ($p < 0.05$) than in the ´Azufrado´ variety, and in both varieties the cooking-mashing-frying treatment caused a decrease HP and NEPA, compared with the cooked-mashed preparations ($p < 0.05$). Again, the decrease in HP and NEPA content in fried samples may be due to the high temperature applied during the cooking

process. Indeed, it has been reported that strong thermal conditions can degrade the HP and NEPA (Sotelo, González-Osnaya, Sánchez-Chinchillas, & Trejo, 2010).

3.3 Bioaccessibility of PC using an in vitro digestion model

The bioaccessibility (%) of PC (Table 3), was significantly different ($p < 0.05$) among the four samples. Differences among samples prepared by the two cooking procedures were also observed during the gastric and intestinal digestion phases ($p < 0.05$). The PC released during these digestion stages were significantly higher ($p < 0.05$) in cooked-mashed samples. The PC-SIF were significantly higher in cooked-mashed samples, but the PC-IIF were higher ($p < 0.05$) in cooked-mashed-fried samples. The gastric digestion promoted a 10-fold increase in the measured PC content compared to the undigested material (Table 2, extractable polyphenols). A similar effect of *in vitro* gastrointestinal digestion has been reported for whole calyces and decoction residues from *Hibiscus sabdariffa* (Mercado-Mercado et al., 2015). It is likely that the low pH of the gastric digestion enhances the abundance undissociated forms of polyphenols, a change that may promote their diffusion from the matrix into the aqueous phase as a consequence of reduced ionic interactions (Bohn, 2014). During the next digestion step (intestinal phase), under neutral pH conditions (6.9) and the presence of α-amylase, pancreatin, lipase and bile salts, the interactions between carbohydrates and PC may be weakened, facilitating PC release and bioaccessibility (Pekkinen et al. 2014). The observed increase of PC during intestinal digestion (Table 3) may thus be due to enzymatic action. Regarding the bean varieties studied, it is interesting to note that ´Negro Jamapa´ showed significantly lower overall bioaccessibility than ´Azufrado´ despite undigested ´Negro Jamapa´ beans contain more TSP (Table 2). According to this, Shahidi et al. (2019) reported that it is most important to know how much PC is bioaccessible and bioavailable than the initial content due no all the

307 initial PC content will be bioaccessible. Some interactions may occur between 'Negro
308 Jamapa' nutrients (dietary fiber, protein) and PC (Pekkinen et al. 2014) that decreased the
309 PC bioaccessibility.
310 It was observed that including a frying step in the bean processing decreased the
311 bioaccessibility of PC, probably due to the association of PC with the IIF. It is known that
312 differences in the type and chemical structure of the PC, as well as cooking conditions, are
313 important factors that may modify the bioaccessibility of PC. These can affect the
314 hydrophobic non-covalent interactions that may occur between the PC and other
315 components of the food matrix (Bohn, 2014).
316 Nevertheless, the decrease in the bioaccessibility of PC in fried samples may be
317 consequence of interactions between PC-lipids and PC-IF. Considering that the IIF-PC
318 associated to the IIF and the content of TIF in cooked-mashed-fried samples were higher (p
319 < 0.05) than in the cooked-mashed preparations, it could be concluded that the decrease
320 observed in the bioaccessibility of PC in the fried samples was probably due to the
321 association of PC with the IF. Besides, the lipid content is higher in fried samples. Then,
322 the presence of water-soluble mixed micelles (formed in the presence of bile salts and
323 lipids) with PC, which may be also interacting with protein (Bohn, 2014), may have led to
324 decreased bioaccessibility of PC. Additionally, Sugiyama et al. (2007) reported that
325 procyanidins and other PC inhibited the lipase activity and decreased the triglycerides
326 absorption. Therefore, extrapolating current observations on the decreased bioaccessibility
327 of PC in fried samples, an increased fecal excretion of lipids could be expected after regular
328 ingestion of fried beans, a hypothesis that should be tested in *in vivo* studies.
329 Overall, present *in vitro* data suggest that about 50% of PC in the cooked-mashed samples
330 and 60-70% in the cooked-mashed-fried preparations would reach the colon unaltered

under *in vivo* conditions. It should be highlighted that this PC fraction might exert health-beneficial effects due to their gut microbial conversion capacity, improving the antioxidant status in the colon (Gong, Chin, Zhang, Wang, & Sun, 2018).

3.4 *In vitro* PC release kinetics

The evaluation of the *in vitro* kinetics of release provides an estimate of the time needed for PC to be released from the food matrix (Blancas-Benítez et al., 2018); the content of PC released during the treatments and the corresponding *Vf* values are shown in Fig. 2. *Vf* represents the time required for the amylase to hydrolyze the glycosidic bonds and release the PC from the digestible carbohydrates (Mercado-Mercado, Montalvo-González, González-Aguilar, Alvarez-Parrilla, & Sáyago-Ayerdi, 2017). CFN sample showed from minute 90 onwards, a higher ($p < 0.05$) release than CN sample; a similar tendency – although not significant- was observed for CFA sample as compared with CA. Moreover, cooked-mashed fried samples showed higher final rates compared to cooked-mashed samples (Fig. 2). This result, according with the above discussed behavior of PC during frying, would reflect the fraction of PC that is being released and that did not form complexes with lipids. However, at 180 min, the PC released in mashed-fried samples decreased while in the mashed samples continued to increase, with CFN always showing the highest bioaccesibility.

The particular PCs released during *in vitro* simulated human digestion, as identified by HPLC-DAD-MS, are shown in Table 4. In particular, three types of compounds were tentatively identified in all the samples, cooking processes and digestion times, they were phenolic acid, chlorogenic acid, and two flavonols, kaempferol 3-*O*-glucoside and quercetin 3-*O*-glucoside. Chlorogenic acid was the main PC released for all samples.

354 Large amounts of chlorogenic acid have been recorded in raw bean extracts (Huber et al.
355 2016). Our observation also agrees with some of the main flavonoids and phenolic acids
356 reported in common beans (Chávez-Mendoza & Sánchez, 2017).
357 In general, major significant differences ($p < 0.05$) were mostly observed between the
358 varieties and not between the cooking processes (Table 4). In particular, ´Negro Jamapa´
359 samples exhibited a higher ($p < 0.05$) PC content than ´Azufrado´ samples, which agrees
360 with TSP results (Fig. 2). Our data also indicate a not always significant tendency for
361 higher PC contents in cooked-mashed-fried preparations than in the cooked-mashed
362 samples, particularly for ´Azufrado´ variety, an observation that also agrees with the TSP
363 results. Regarding the changes with time, chlorogenic acid exhibited a maximum release at
364 60 min for most samples, while kaempferol-3-*O*-glucoside peaked at 30 min. In contrast,
365 the behavior of quercetin-3-*O*-glucoside was not so clear, with a maximum ranging from 30
366 min in CA sample to 120 min in CN. Considering the three PC together, the highest levels
367 were obtained at 30 and 60 min, while for further times the concentrations tended to
368 maintain constant. This could mean that the passive transport mechanism remained constant
369 throughout the intestinal digestion stage. Besides, Tovar, Sáyago-Ayerdi, Peñalver,
370 Paredes-López, and Bello-Pérez (2003) reported that the starch-containing cells in beans
371 possess rigid cell walls and that this mechanical resistance is still observed in boiled and
372 mildly homogenized beans, a characteristic that may limit the release of PC. However,
373 during the kinetics of release evaluation the PC may be interacting with the dietary fiber in
374 the rigid cell walls, and this interaction may allow a constant release of the PC.
375 Additionally, Takeoka et al. (1997) characterized the anthocyanins profile of common
376 black beans identifying a number of compounds such as delphinidin-3-glucoside,
377 petunidin-3-glucoside, and malvidin-3-glucoside. In this study, anthocyanins were not

detected in any of the samples, as observed in a previous study of pressure cooked of common black beans (Moreno-Jiménez et al. 2015). It should be highlighted that the PC identified during the *in vitro* release kinetics were those expected to be bioaccessible. In contrast to previous studies performed with raw beans, data provided here with a model that emulates the physiological processes, contribute to increase the knowledge on the type of PC that may be bioaccessible *in vivo* and, therefore, potentially absorbed into the bloodstream and exert biological activities. Indeed, here identified PCs have been reported to show different activities regarding inflammation, vasodilation or cell proliferation (~~Chávez-Mendoza & Sánchez, 2017;~~ Lee, Lee, Kim, & Kim, 2016). Our results, together with those from future investigations on the metabolic fate of PC in cooked beans may help in the design on new studies and disease-preventing strategies based on health-related properties of legumes.

CONCLUSIONS

The cooking conditions affected the nutritional composition and the *in vitro* bioaccessibility of PC of two common bean varieties. In particular, frying caused a decrease of TSP content but at the same time it led to an increase in TIF content. The apparent association of PC to the IIF observed during the *in vitro* bioaccessibility evaluation, caused a decrease in PC bioaccessibility but it did not affect the kinetics of their release.

A sustained PC release for at least 150 min was observed in all samples, which might imply a prolonged circulation of PC in the blood, following a bean meal. Overall, this study provides new data on the potential bioaccessibility of PC in ´Azufrado´ and ´Negro Jamapa´ varieties after gastrointestinal conditions and after single pressure cooking an after a post-cooking frying step, a traditional Mexican culinary habit for bean consumption. Moreover, an estimate of about 50% of PC in the cooked bean preparations and 60-70% in

the cooked-fried samples has the ability to reach the human colon, contributing to the antioxidant potential in the large intestine. These results should be considered in future studies on the biological activities of this legume.

Conflict of interest

The authors report no conflicts of interest

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Authors' contribution

Authors' contribution. Data curation, writing and editing of the manuscript: Tovar Juscelino, Pérez-Jiménez, Jara, Bello-Pérez, Luis A.

Conceptualization, formal analysis, data curation, original draft preparation, and corrections: Cárdenas-Castro, A.P.

Writing, review, supervision, editing, and final revision: Sáyago-Ayerdi, S.G.

REFERENCES

Alvarez-Parrilla, E., de la Rosa, L.A., Amarowicz, R., & Shahidi, F. (2010). Antioxidant activity of fresh and processed Jalapeno and Serrano peppers. *Journal of Agricultural and Food Chemistry, 59(1),* 163-173.

AOAC. (2005). Association of Official Analytical Chemistry. USA.

Bello-Pérez, L.A., Flores-Silva, P.C., Agama-Acevedo, E. & Tovar, J. (2019). Starch digestibility: past, present and future. *Journal of the Science of Food and Agriculture* (in press). https://doi.org/10.1002/jsfa.8955

Blancas-Benítez, F.J., Mercado-Mercado, G., Quirós-Sauceda, A.E., Montalvo-González, E., González-Aguilar, G.A., & Sáyago-Ayerdi, S.G. (2015). Bioaccessibility of polyphenols associated with dietary fiber and *in vitro* kinetics release of polyphenols in Mexican 'Ataulfo' mango (*Mangifera indica* L.) by-products. *Food & Function, 6,* 859-868.

Blancas-Benítez, F.J., Pérez-Jiménez, J., Montalvo-González, E., González-Aguilar, G.A., & Sáyago-Ayerdi, S.G. (2018). *In vitro* evaluation of the kinetics of the release of phenolic compounds from guava (*Psidium guajava* L.) fruit. *Journal of Functional Foods, 43,* 139-145.

Bohn, T. (2014). Dietary factors affecting polyphenol bioavailability. Nutr Rev 72:429-452.

Chávez-Mendoza, C., & Sánchez, E. (2017). Bioactive compounds from Mexican varieties of the common bean (*Phaseolus vulgaris*): Implications for Health. *Molecules, 22,* 1-32.

Gan, R.Y., Wang, M. F., Lui, W. Y., Wu, Y., Wu, K., Dai, S. H., Sui, Z.Q., & Corke, H. (2017). Diversity in antioxidant capacity, phenolic contents, and flavonoid contents of 42 edible beans from China. *Cereal Chemistry, 94,* 291-297.

Gong, L., Chi, J., Zhang, Y., Wang, J., & Sun, B. (2018). *In vitro* evaluation of the bioaccessibility of phenolic acids in different whole wheats as potential prebiotics. *LWT-Food Science and Technology, 100,* 435-443.

Goñi, I., García-Diz, L., Mañas, E., & Saura-Calixto, F. (1996). Analysis of resistant starch: a method for foods and food products. *Food Chemistry, 56,* 445-449.

446 Granfeldt, Y., Björck, I., Drews, A., & Tovar, J. (1992). An *in vitro* procedure based on
447 chewing to predict metabolic response to starch in cereal and legume products.
448 *American Journal of Clinical Hypnosis, 59,* 777S-777S.
449 Hartzfeld, P.W., Forkner, R., Hunter, M.D., & Hagerman, A.E. (2002). Determination of
450 hydrolyzable tannins (gallotannins and ellagitannins) after reaction with potassium
451 iodate. *Journal of Agricultural and Food Chemistry, 50,* 1785-1790.
452 Hernández-Salazar, M., Osorio-Diaz, P., Loarca-Piña, G., Reynoso-Camacho, R., Tovar, J.,
453 & Bello-Pérez, L.A. (2010). *In vitro* fermentability and antioxidant capacity of the
454 indigestible fraction of cooked black beans (*Phaseolus vulgaris* L.), lentils (*Lens*
455 *culinaris* L.) and chickpeas (*Cicer arietinum* L.). *Journal of the Science of Food*
456 *and Agriculture, 90,* 1417-1422.
457 Holm, J., Björck, I., Drews, A., & Asp, N.G. (1986). A rapid method for the analysis of
458 starch. *Stärke, 38,* 224-226.
459 Huber, K., Brigide, P., Bolis, E., & Canniatti-Brazaca, S. (2016). Phenolic acid, flavonoids
460 and antioxidant activity of common brown beans (*Phaseolus vulgaris* L.) before and
461 after cooking. *Journal of Nutrition & Food Sciences, 6,* 1-7.
462 Lee, J., Lee, J., Kim, S.J., & Kim, J.H. (2016). Quercetin-3-O-glucoside suppresses
463 pancreatic cancer cell migration induced by tumor-deteriorated growth factors *in*
464 *vitro*. *Oncology Reports, 35,* 2473-2479.
465 Lin, L.Z., Harnly, J.M., Pastor-Corrales, M.S., & Luthria, D.L. (2008). The polyphenolic
466 profiles of common bean (*Phaseolus vulgaris* L.). *Food Chemistry, 107,* 399-410.
467 Jakobek, L. (2015). Interactions of polyphenols with carbohydrates, lipids and proteins.
468 *Food Chemistry, 175,* 556-567.

469 Mercado-Mercado, G., Blancas-Benítez, F.J., Velderrain-Rodríguez, G.R., Montalvo-
470 González, E., González-Aguilar, G.A., Alvarez-Parrilla, E., & Sáyago-Ayerdi, S.G.
471 (2015). Bioaccessibility of polyphenols released and associated to dietary fibre in
472 calyces and decoction residues of Roselle (*Hibiscus sabdariffa* L.). *Journal of*
473 *Functional Foods, 18,* 171-181.
474 Mercado-Mercado, G., Montalvo-González, E., González-Aguilar, G.A., Álvarez-Parrilla
475 E., & Sáyago-Ayerdi, S.G. (2017). Ultrasound-assisted extraction of carotenoids
476 from mango (*Mangifera indica* L. ´Ataulfo´) by-products on *in vitro*
477 bioaccessibility. *Food Bioscence, 21,* 125-131.
478 Montreau, F. (1972). Sur le dosage des composés phénoliques totaux dans les vins par la
479 methode Folin-Ciocalteau. *Connaiss Vigne Vin*, *24,* 397-404.
480 Moreno-Jiménez, M.R., Cervantes-Cardoza, V., Gallegos-Infante, J.A., González-Laredo,
481 R.F., Estrella, I., García-Gasca, T.D.J., …& Rocha-Guzmán, N.E. (2015). Phenolic
482 composition changes of processed common beans: Their antioxidant and anti-
483 inflammatory effects in intestinal cancer cells. *Food Research International, 76,* 79-
484 85.
485 Pekkinen, J., Rosa, N.N., Savolainen, O.I., Keski-Rahkonen, O., Mykkänen, H., Poutanen,
486 K., & Hanhineva, K. (2014). Disintegration of wheat aleurone structure has an
487 impact on the bioavailability of phenolic compounds and other phytochemicals as
488 evidenced by altered urinary metabolite profile of diet-induced obese mice.
489 *Nutrition & Metabolism, 11*, 1-9.
490 Pérez-Burillo, S., Rufián-Henarez, J.A., & Pastoriza, S. (2018). Effect of home cooking on
491 the antioxidant capacity of vegetables: Relationship with Maillard reaction

indicators. *Food Research International.* https://doi.org/10.1016/j.foodres.2018.12.007

Pérez-Jiménez, J., Arranz, S., Tabernero, M., Díaz-Rubio, M.E., Serrano, J., Goñi, I., & Saura-Calixto, F. (2008). Updated methodology to determine antioxidant capacity in plant foods, oils and beverages: Extraction, measurement and expression of results. *Food Research International, 41,* 274-285.

Reed, J.D., McDowell, R.T., Van Soest, P.J., & Horvath, P.R. (1982). Condensed tannins: a factor limiting the use of cassava forage. *Journal of the Science of Food and Agriculture, 33,* 213-220.

Rehinan, Z.U., Rashid, M., & Shah, W. (2004). Insoluble dietary fiber components of food legumes as affected by soaking and cooking processes. *Food Chemistry, 85,* 245-249.

Saura-Calixto, F., García-Alonso, A., Goñi, I., & Bravo, L. (2000). *In vitro* determination of the indigestible fraction in foods: an alternative to dietary fiber analysis. *Journal of Agricultural and Food Chemistry, 48,* 3342-3347.

Sáyago-Ayerdi, S., Tovar, J., Osorio-Diaz, P., Paredes-López, O., & Bello-Pérez, L.A. (2005). *In vitro* starch digestibility and predicted glycemic index of corn tortilla, black beans, and tortilla−bean mixture: Effect of cold storage. *Journal of Agricultural and Food Chemistry*, *53*, 1281-1285.

Shahidi, F., Varatharajan, V., Oh, W.Y., & Peng, H. (2019). Phenolic compounds in agri-food by-products, their bioavailability and health effects. *Journal of Food Bioactives, 5,* 57-119.

514 Silva-Cristobal, L., Osorio-Díaz, P., Tovar, J., & Bello-Pérez, L. (2010). Chemical
515 composition, carbohydrate digestibility, and antioxidant capacity of cooked black
516 bean, chickpea, and lentil Mexican varieties. *CyTA–Journal of Food, 8,* 7-14.
517 Sotelo, A., González-Osnaya, L., Sánchez-Chinchillas, A., & Trejo, A. (2010). Role of
518 oxate, phytate, tannins and cooking on iron bioavailability from foods commonly
519 consumed in Mexico. *Internation Journal of Food Sciences and Nutrition, 61,* 29-
520 39.
521 Sugiyama, H., Akazome, Y., Shoji, T., Yamaguchi, A., Yasue, M., Kanda, T., & Ohtake,
522 Y. (2007). Oligomeric procyanidins in apple polyphenol are main active
523 components for inhibition of pancreatic lipase and triglyceride absorption. *Journal*
524 *of Agricultural and Food Chemistry*, *55,* 4604-4609.
525 Takeoka, G.R., Dao, L.T., Full, G.H., Wong, R.Y., Harden, L.A., Edwards, R.H., &
526 Berrios, J.D.J. (1997). Characterization of black bean (*Phaseolus vulgaris* L.)
527 anthocyanins. *Journal of Agricultural and Food Chemistry*, *45,* 3395-3400.
528 Tovar, J., Nilsson, A., Johansson, M., & Björck, I. (2014). Combining functional features
529 of whole-grain barley and legumes for dietary reduction of cardiometabolic risk: a
530 randomised cross-over intervention in mature women. *European Journal of*
531 *Nutrition, 111,* 706-714.
532 Tovar, J., Sáyago-Ayerdi, S., Peñalver, C., Paredes-López, O., & Bello-Pérez, L.A. (2003).
533 In vitro starch hydrolysis index and predicted glycemic index of corn tortilla, black
534 beans (*Phaseolus vulgaris* L.), and Mexican "taco". *Cereal Chemistry, 80,* 533-535.
535 Wang, N., Hatcher, D., Tyler, R., Toews, R., & Gawalko, E. (2010). Effect of cooking on
536 the composition of beans (*Phaseolus vulgaris* L.) and chickpeas (*Cicer arietinum*
537 L.). *Food Research International, 43,* 589-594.

538 Woolnough, J.W., Bird, A.R., Monro, J.A., & Brennan, C.S. (2010). The effect of a brief
539 salivary α-amylase exposure during chewing on subsequent in vitro starch digestion
540 curve profiles. *International Journal of Molecular Sciences, 11,* 2780-2790.
541 Xu, B., & Chang, S. (2008). Total phenolic content and antioxidant properties of eclipse
542 black beans (*Phaseolus vulgaris* L.) as affected by processing methods. *Journal of*
543 *Food Science, 73,* H19-H27.

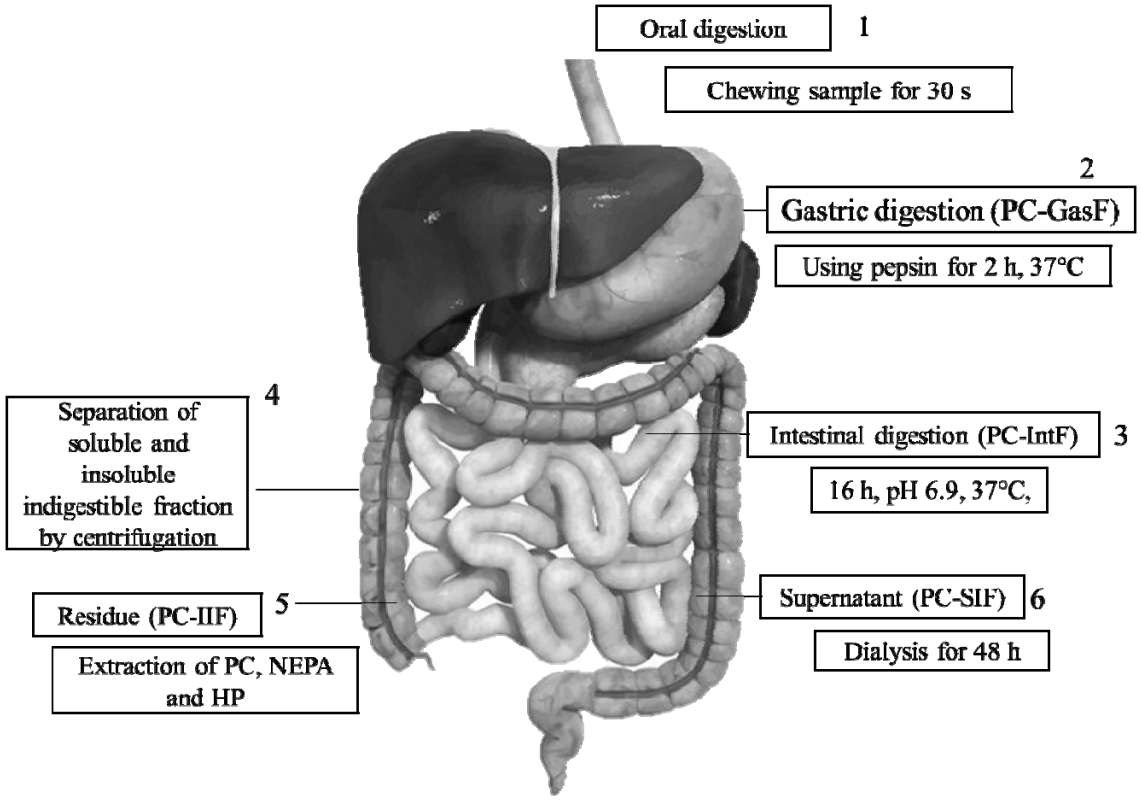

FIGURE 1

Bioaccessibility of phenolic compounds methodology. Step 1: Oral digestion by chewing of freshly processed sample. Step 2: Pepsin, 37°C, 2h; PC released during gastric digestion; PC-GasF= the PC present in the gastric fraction. Step 3: Pancreatin and amylase for grounded samples, 6 h; pancreatin, amylase, bile salts and lipase for fried samples; PC-IntF= the PC released on the intestinal fraction. Step 4: Indigestible fraction separation by centrifugation. Step 5: Non-bioaccessible PC associated to insoluble indigestible fraction as

a sum of TSP= Total soluble polyphenols (organic aqueous extraction); NEPA= non-extractable proanthocyanidins and PH= hydrolysable polyphenols (chemical hydrolysis); PC-IIF= the PC associated with insoluble indigestible fraction. Step 6: Dialysis 48 h, non-bioaccessible PC associated to soluble indigestible fraction; PC-SIF= the PC associated with soluble indigestible fraction.

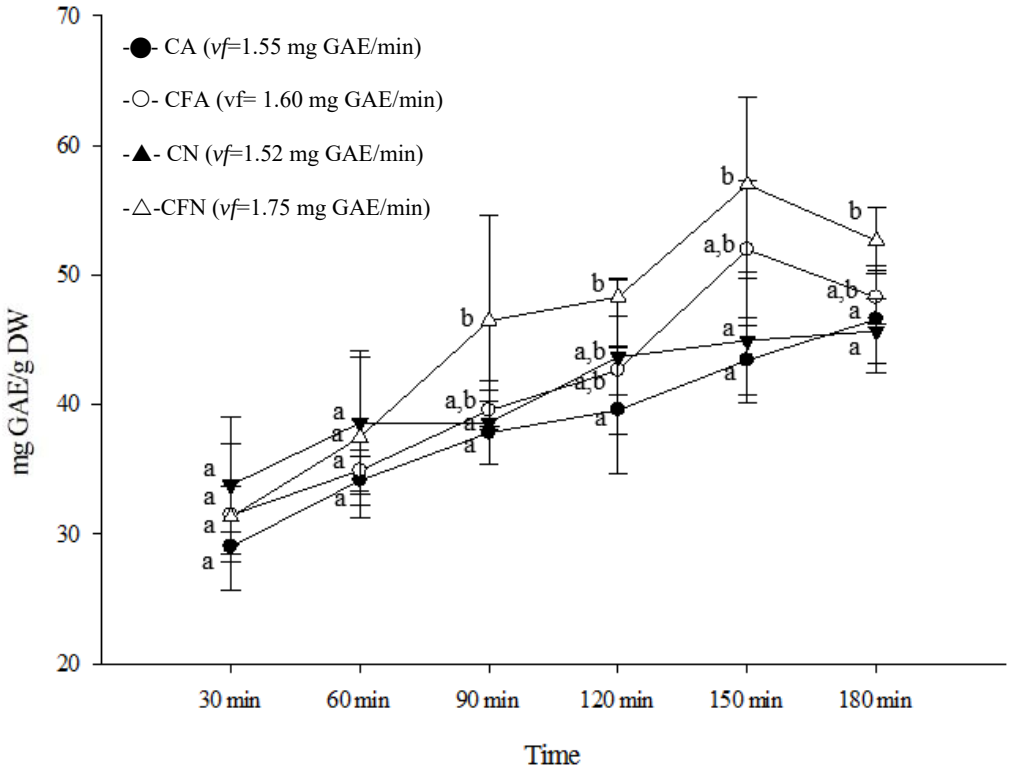

FIGURE 2 *In vitro* kinetics of the TSP (mg GAE/g DW) release from two common bean *(Phaseolus vulgaris* L.*)* varieties pressured cooked (mashed and mashed-fried). Different lowercase letters at the same time point indicate significant difference (n=3) ($p<0.05$). CA, cooked-mashed 'Azufrado' (●); CFA, cooked-fried 'Azufrado' (○); CFN, cooked-fried 'Negro Jamapa' (△); CN, cooked-mashed 'Negro Jamapa' (▲).

TABLE 1 Chemical composition of cooked-mashed ´Azufrado´ (CA), and cooked-mashed ´Negro Jamapa´ (CN), cooked-mashed-fried ´Azufrado' (CFA) and 'cooked-mashed-fried´ ´Negro Jamapa´ (CFN) of common bean varieties (g/100 g DW)[1].

Parameter	CA	CFA	CN	CFN
Moisture	71.49 ± 0.24^{b}	60.31 ± 1.09^{a}	72.97 ± 0.11^{b}	60.32 ± 1.40^{a}
Ash	5.16 ± 0.13^{c}	4.38 ± 0.06^{b}	4.44 ± 0.04^{b}	3.68 ± 0.11^{a}
Fat	2.48 ± 0.12^{a}	13.01 ± 0.24^{b}	2.17 ± 0.35^{a}	17.84 ± 0.15^{c}
Protein[2]	25.10 ± 0.53^{c}	24.88 ± 0.34^{bc}	23.77 ± 0.85^{a}	23.90 ± 0.28^{ab}
Available starch	47.02 ± 0.87^{b}	44.85 ± 0.65^{ab}	43.97 ± 1.27^{a}	45.02 ± 2.36^{ab}
Resistant starch	4.32 ± 0.06^{a}	4.96 ± 0.10^{b}	5.45 ± 0.12^{c}	5.95 ± 0.14^{d}
Total indigestible fraction	28.70 ± 1.89^{a}	41.18 ± 0.35^{c}	33.04 ± 0.36^{b}	45.32 ± 0.32^{d}
Soluble indigestible fraction	4.41 ± 0.52^{a}	4.32 ± 0.63^{a}	4.61 ± 0.62^{a}	4.46 ± 0.21^{a}
Insoluble indigestible fraction	24.38 ± 2.52^{a}	36.76 ± 0.38^{c}	28.58 ± 0.14^{b}	40.70 ± 0.68^{d}

[1]Values represent mean ± SD (n=3). Different lowercase letters in the same row indicate significant difference ($p<0.05$). [2]6.25=Conversion factor.

TABLE 2 Phenolic compounds content and associated antioxidant capacity of cooked-mashed ´Azufrado´ (CA), cooked-mashed ´Negro Jamapa´ (CN), cooked-mashed-fried´Azufrado' (CFA) and 'cooked-mashed-fried´ ´Negro Jamapa´ (CFN) of common bean varieties

Parameter	CA	CFA	CN	CFN
Extractable polyphenols				
TSP[2]	2.7 ± 0.1[b]	1.7 ± 0.1[a]	3.7 ± 0.3[c]	2.9 ± 0.1[b]
Antioxidant capacity				
ABTS[3]	63.14 ± 1.91[a]	66.09 ± 4.78[a]	95.46 ± 6.59[b]	84.72 ± 1.88[c]
FRAP[3]	74.57 ± 1.44[c]	61.68 ± 1.44[a]	88.31 ± 0.30[d]	69.83 ± 0.39[b]
Non-extractable polyphenols				
HP[4]	13.55 ± 0.48[a]	11.70 ± 1.06[b]	18.72 ± 0.49[c]	14.12 ± 0.56[a]
NEPA[5]	5.80 ± 0.37[b]	3.87 ± 0.19[a]	12.7 ± 0.75[d]	8.53 ± 0.02[c]

[1]Values represent mean ± SD (n=3). Different lowercase letters in the same row indicate significant difference ($p<0.05$). [2]TSP; Total soluble polyphenols (mg GAE/g DW). [3]Trolox equivalents (mmol TE/g DW). ABTS (2,2´-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), FRAP (ferric reducing/antioxidant power). [4]HP= hydrolyzable polyphenols (mg GAE/g DW). [5]NEPA= non-extractable proanthocyanidins (mg/g DW).

TABLE 3 *In vitro* bioaccessibility (BA) of phenolic compounds (PC) of cooked-mashed ´Azufrado´ (CA), and cooked-mashed ´Negro Jamapa´ (CN), cooked-mashed-fried 'Azufrado' (CFA) and 'cooked-mashed-fried´ ´Negro Jamapa´ (CFN) of common bean varieties two common bean varieties (g/100 g DW)[1].

Parameter	CA	CFA	CN	CFN
PC-GasF	2.04 ± 0.28[b]	1.67 ± 0.06[a]	2.00 ± 0.44[b]	1.46 ± 0.06[a]
PC-IntF	3.53 ± 0.23[a]	2.75 ± 0.12[b]	3.84 ± 0.38[a]	2.61 ± 0.27[b]
PC-SIF	1.22 ± 0.07 [a]	0.98 ± 0.04[c]	1.37 ± 0.09[b]	0.96 ± 0.04[c]
PC-IIF	0.77 ± 0.07 [a]	1.80 ± 0.04[c]	1.22 ± 0.09[b]	2.64 ± 0.04[d]
%BA[2]	53.58 ± 2.27[a]	39.36 ± 2.08[c]	48.44 ± 3.53[b]	31.00 ± 1.17[d]

[1]Values represent mean ± SD (n=3). Different lowercase letters in the same row indicate significant difference ($p<0.05$). [2]$\%BA = \frac{(PC-Int)-(PC-SIF)}{(PC-Int)+(PC-IIF)} x\ 100$; PC-GasF= the PC present in the gastric fraction, PC-IntF= the PC released on the intestinal fraction, PC-IIF= the PC associated with insoluble indigestible fraction, PC-SIF= the PC associated with soluble indigestible fraction, %BA=percentage of the bioaccessibility

TABLE 4 Tentative phenolic compounds profile in each time period of phenolic compounds release kinetics of cooked-mashed ´Azufrado´ (CA), and cooked-mashed ´Negro Jamapa´ (CN), cooked-mashed-fried´Azufrado' (CFA) and 'cooked-mashed-fried´ ´Negro Jamapa´ (CFN) of common bean varieties [1].

Time (min)	Compound	m/z	Rt (min)	MS Area[2]		
				CA	CFA	CN
30	Chlorogenic acid	353.31(-)	3.00	1,090.8 ± 53.3^{Aa}	4,082.2 ± 284.3^{Ab}	8,830.1 ± 602.8^{Cc}
	Kaempferol 3-O-glucoside	449.38(+)	3.05	14,760.9 ± 1,037.1^{Bab}	12,687.8 ± 1,171^{Aa}	17,874.9 ± 2,154.5^{Ab}
	Quercetin-3-O-glucoside	465.09(+)	3.19	22,446.55 ± 574.7^{Ca}	21,175.2 ± 456.4^{Ba}	25,278.4 ± 155.9^{Ab}
60	Chlorogenic acid	353.31(-)	3.00	3,344.5 ± 141.3^{Ba}	5,889.3 ± 430.3^{Bb}	6,253.1 ± 270.8^{ABb}
	Kaempferol 3-O-glucoside	449.38(+)	3.05	9,222.8 ± 1,407.7 ^{Aa}	11,583.4 ± 2,306.9^{Aa}	25,625.4 ± 4,549.6^{Bb}
	Quercetin-3-O-glucoside	465.09(+)	3.19	18,258.05 ± 492.6^{ABa}	21,537.4 ± 109.2^{Bb}	30,019.7 ± 859.4^{Bc}
90	Chlorogenic acid	353.31(-)	3.00	1,411.5 ± 205.2^{Aab}	4,278.6 ± 410.8^{Ab}	6,258 ± 711^{ABc}
	Kaempferol 3-O-glucoside	449.38(+)	3.05	9600.6 ± 1,329.1 ^{Aa}	15,242.6 ± 1,607.5^{Ab}	30,689 ± 4,168.5^{Bc}
	Quercetin-3-O-glucoside	465.09(+)	3.19	18,031 ± 540.5^{ABa}	23,509.9 ± 314.7^{Cb}	30,152.6 ± 1,383.9^{Bc}
120	Chlorogenic acid	353.31(-)	3.00	5,261.4 ± 350.9^{Cbc}	3,572.1 ± 329.5^{Aa}	6,328.8 ± 347.6^{ABc}
	Kaempferol 3-O-glucoside	449.38(+)	3.05	9,912.1 ± 1,576.5 ^{Aa}	12,631.3 ± 2,938.5^{Aa}	28,508.5 ± 4,684.9^{Bb}
	Quercetin-3-O-glucoside	465.09(+)	3.19	16,185.9 ± 1,465.3^{Aa}	19,452.2 ± 285.6^{Ba}	31,120.4 ± 1,224.2^{Bb}
150	Chlorogenic acid	353.31(-)	3.00	4,791.4 ± 113^{Ca}	6,022.6 ± 491.9^{Bb}	4,955.5 ± 480.2^{Aab}
	Kaempferol 3-O-glucoside	449.38(+)	3.05	9,627.75± 204.8 ^{Aa}	11,788.4 ± 1,666.9^{Aa}	30,342.5 ± 3,352.7^{Bc}
	Quercetin-3-O-glucoside	465.09(+)	3.19	17,246 ± 921.9^{Aa}	16,958.4 ± 387.9^{Aa}	29,386.6 ± 594.3^{Bb}
180	Chlorogenic acid	353.31(-)	3.00	2,996.1 ± 489.7^{Ba}	5,808.8 ± 412.6^{Bb}	6,680.5 ± 422.3^{Bb}

Kaempferol 3-O-glucoside	449.38(+)	3.05	10,336.5 ± 1,547.4 ^{Aa}	11,349.8 ± 1,674.4^{Aa}	29,626.9 ± 2,125.9^{Bc}
Quercetin-3-O-glucoside	465.09(+)	3.19	20,371.4 ± 1,313.5^{BCa}	21,867.73 ± 2,131^{BCa}	30,043.9 ± 154.7^{Bb}

Different uppercase letter indicate significant difference between times of kinetics for each compound and each sample, different lower case letters in the same row indicate significant difference between samples on each kinetic time ($p < 0.05$).[1]Values represent mean ± standard deviation (n=3); [2]Arbitrary units.